

## The Biological and Systematic Significance of Red Fecal and Meconial Pigments in Butterflies: A Review with Special Reference to the Pieridae

Arthur M. Shapiro

Department of Zoology, University of California, Davis, California 95616

**Abstract.** Red fecal pellets produced just before pupation and red meconium voided by teneral adults are distributed in specific butterfly lineages and may be useful in taxonomy. The red pigments are ommochromes; a brief review of their biosynthesis and physiology is presented. Abnormal production of red ommochromes may follow various disease processes and injuries in Pierid early stages.

The topic of "red rain" was introduced to several generations of American Lepidopterists by W. J. Holland, who reproduced on pages 299-303 of *The Butterfly Book* (1898) an essay on the subject by Frank Cowan. Those who rear Nymphalini are familiar with the bright red meconium which inspired superstitious terror among the European peasantry, but the literature treating the chemistry and physiology of this phenomenon is generally unknown to Lepidopterists, and the potential significance of red excretory pigments in butterfly systematics remains virtually unremarked. This paper presents a brief survey of these subjects, with some hitherto unpublished information on the phenomenon as it occurs in various Pieridae.

Meconium—the material voided upon or shortly after eclosion by a teneral (immature adult) insect—is often pigmented; the color may be characteristic of the taxon. Red meconia are best known in the Nymphalini, in the "tortoiseshell" group of genera variously known as *Nymphalis*, *Aglais*, *Inachis*, *Vanessa*, etc. (In this paper American rather than European taxonomic conventions will be followed.) They also occur in some other Nymphalid genera which are not closely related, such as *Euphydryas* and *Charaxes*. There appear to be very few mentions of fecal or meconial colors in the literature of butterfly breeding. According to one anonymous reviewer of this paper, red meconia occur in the Papilionid genera *Parnassius* (*Tadumia*) and *Allancastris*; *P. clodius sol* Bryk and Eisner from Nevada County, California, has pink meconium (Shapiro, unpublished). In the Pieridae they are widespread but not universal in the Pierini and Anthocharini (= Euchloini).

## Chemistry and Normal Biosynthesis

Meconium is regarded as the waste products of metamorphosis. There are two phases of meconium production in the teneral adult (Lafont and Penetier, 1975). In the first, red pigments—ommochromes—and uric acid are excreted; the second, which is essentially colorless, contains allantoic acid. Many other compounds have been identified in meconium, but we are concerned primarily with the ommochromes, a group of pigments best known from insect eyes, whence the name. They were reviewed in depth by Linzen (1974), from which much of this section is abstracted. Ommochromes (Fig. 1) are degradation products of the amino

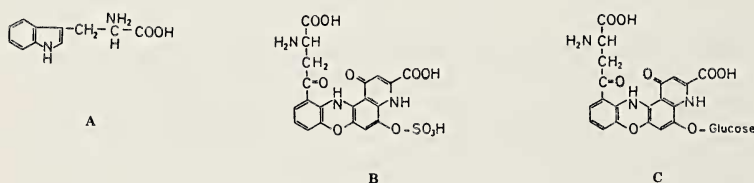


Fig. 1. Structural formulae of the precursor amino acid, tryptophan (A) and its derivatives ommatin D (B) and rhodommatin (C), the two common red excreory pigments found in Pierid and Nymphalid meconia. The biosynthetic pathways are given in detail by Linzen, p. 123.

acid tryptophan. In the Nymphalini two ommochromes, ommatin D and rhodommatin, occur as wing pigments as well as in meconium. The same pigments have been recovered from the meconia of the Palearctic Pierids *Aporia crataegi* L. and *Pieris brassicae* L., in the latter only in very small amounts. Ommochromes seem unreported from Pierid wings. They were first obtained in large quantities by Becker (1942) and Butenandt *et al.* (1954) from the meconium of the European Small Tortoiseshell, *Nymphalis (Aglais) urticae* L. In the Red Admiral, *Vanessa atalanta* L., up to 130  $\mu\text{g}$  of rhodommatin and 115  $\mu\text{g}$  of ommatin D have been recovered from the meconium of a single animal; in *A. crataegi* these values are 244  $\mu\text{g}$  and 225  $\mu\text{g}$  respectively. Linzen summarizes the history of these compounds in the insect as follows (pages 176-177):

These ommochromes are formed early in metamorphosis. They make their first appearance at the time when the larvae leave the food and crawl about to find a suitable place for pupation. In (*Nymphalis*) *urticae*, orange pigment appears in the midgut wall at the time when the larva is spinning the small web to hang itself up.

Twelve hours later, at the time of pupation, the gut is filled with red fluid containing about 50  $\mu\text{g}$  each of rhodommatin and ommatin D.

More is known in certain moths. In *Ptychopoda seriata* Schrk. (Geometridae) and *Cerura vinula* L. (Notodontidae), studied by authors cited

in Linzen's review, ommochromes appear twice during development, as they do in Pieridae. First they appear in the Malpighian tubules, which excrete them—contributing to a red color in the last few fecal pellets prior to pupation. Later (after pupation), during the reconstruction of the tissues, ommochromes appear in vacuoles of the new midgut epithelium and are released into the lumen of the gut, where they contribute to the first meconium of the teneral adult.

The only definite adaptive function of these releases is the removal of excess tryptophan, but they may serve subsidiary functions as well. In many Lepidoptera meconium is retained by the teneral adult until it is ready for flight, or even longer, but it may be released early if the animal is disturbed. Such releases are often forceful, with a definite potential for defense against predation. Meconium has not, to my knowledge, been examined for repellent or noxious properties. It is perhaps not too far-fetched that the blood-red color of first meconium forcibly voided in such circumstances by the otherwise defenseless teneral adult may be alarming or at least distracting to vertebrate predators having color vision.

### Distribution in the Pieridae

In the Holarctic pierine fauna the last few (1-4) fecal pellets produced before pupation, and the first meconium of the adult, are red in certain lineages and not in others; the two seem always to be coupled. These facts suggest that they may be useful characters for phylogenetic reconstruction and higher classification. The only previous use of meconium in taxonomy appears to be by DeBach *et al.* (1978), working on *Aphytis* spp. (Hymenoptera, Aphelinidae).

Ommochrome-excreting Holarctic pierines belong to the *Pieris* (*Synchlœe*) *callidice* Hbn. species-group, all of whose taxa thus far examined (*callidice*, *protodice* Bdv. & LeC., *occidentalis* Reak., *nelsoni* Edw.) show the phenomenon. Meconia are unreported in the formal literature for the closely related subgenus *Pontia* and for the enigmatic *P. (Pontia?) sisymbrii* Bdv., but one of the anonymous reviewers of this paper reports red meconia for the Palearctic *P. (Pontia) daplidice* L. and *glauconome* Klug. No red ommochromes are found in feces or meconium of the *P. rapae* L. and *P. napi* L. species-groups (subgenus *Artogeia*) except under pathological circumstances (see below), and they are present but scarce in *P. (Pieris) brassicae*. The highest levels ever measured occur in *Aporia crataegi* and in some of the South American taxa discussed below.

In the Andean-Patagonian group of Pierid genera, red fecal pellets and meconia seem generally distributed in the *Tatochila-Phulia* series. Taxa reared by me, all of which show the phenomenon, are *Reliquia santamarta* Ackery and the following entities in *Tatochila*: *sterodice* species-group: *sterodice* Stgr., *arctodice* Stghr., *macrodice* Stgr., *vanvolxemii* Capr., *mercedis* Esch.; *xanthodice* species-group: *xanthodice* Lucas; *autodice*



species-group: *autodice* Hbn., *blanchardii* Butl. Quantities are especially large in the *sterodice* group.

In the Anthocharini, red feces and meconia occur in the Holarctic *Euchloe ausonia* species-group: *ausonia ausonia* Hbn., *a. crameri* Butl., and *ausonides* Lucas (several segregates). They are pink in *Anthocharis sara* Lucas and *Euchloe hyantis* Edw. Red excreta are recorded formally in only one Coliadine species; *Eurema hecabe* L. (Fukuda *et al.*, 1972), but are reported by the aforementioned reviewer of this paper to occur in both *Catopsilia* and *Gonepteryx*.

The distribution of red feces and meconia in Pierini and Anthocharini corresponds to the distribution of "red" eggs (Shapiro, 1981). This at least suggests the possibility that the red pigment developed by maturing Pierid eggs might be an ommochrome, since Horowitz (1940) and Horowitz and Baumberger (1941) found that eggs of the marine Echiuroid worm *Urechis* undergo color changes similar to those of Pierids. In this case, the ommochromes function as respiratory pigments; the color change indicates a shift from the oxidized to the reduced state. The well-known color changes of *Euchloe* prepupae (from gray-violet and yellow to pinkish gray in *E. ausonides*, from green to lurid purple in *E. hyantis*) may also be mediated by ommochromes.

The taxonomic distribution of ommochromes as excretory pigments in Pieridae corroborates the conventional phylogenetic interpretation of the Andean-Patagonian fauna (Shapiro, 1980): post-Pliocene derivation from a Holarctic ancestor of the *Synchloe* type. It also supports the electrophoretic evidence of Courtney (1980) and Geiger (1981) indicating a close relationship between *Pontia* and *Synchloe* on one hand and the Anthocharini on the other. This raises the serious question of whether the bizarre Andean *Eroessa* is really an orange-tip at all. It has been considered the most primitive member of that group, but that interpretation would remain tenable only by deriving the Pierini from *Euchloe* or something near it, an unlikely scenario on various grounds.

Phylogenetic interpretation in the Pieridae is complicated by an obvious predisposition to parallelisms and convergences which makes even cladistic reasoning highly unsatisfying. The distribution of excretory ommochromes parallels phenotypic resemblances which have usually been interpreted as superficial and convergent. Both types of characters could have evolved as correlates of dietary specialization (on inflorescences and infructescences); if tryptophan loads are heavier in the diets of these groups than in those of leaf-feeders, physiological parallelism or convergence would be implied. It must be stressed that there is no *a priori* reason why biochemical characters should be less prone to convergence than morphological ones.

#### **Ommochromes in Pathological Contexts**

Linzen (1974, p. 177) observes that ommochromes may be produced

when insects or their tissues "are maltreated so as to disrupt normal metabolic function...it is assumed that under these circumstances the animals draw on their protein reserves as an energy source, thus leading to **an excess of tryptophan.**" Phenomena of this sort are readily observed in Pieridae.

Pierid breeders eventually make the acquaintance of "red dribble disease," a condition of unknown etiology which attacks fourth- and fifth-instar larvae with invariably fatal results. The afflicted larva suddenly stops feeding and begins producing a more or less constant dribble of bright red diarrhea. It never feeds again, but may live for several weeks, shrinking all the while as it consumes its reserves. Most afflicted larvae spin a great deal of silk and may appear to be prepared to pupate, but fail to do so. The condition is associated with etiolated food, with certain Crucifers which are edible but apparently inimical to survival (such as flowering tops, but not rosettes, of the weed *Barbarea vulgaris* R.Br.), and with inbreeding depression in long-term cultures; it does not seem to be infectious or contagious. I have encountered it in cultures of *Pieris* (*Synchlœ*) and of *Tatochila* and—interestingly—of *P. rapae* and *P. napi*, which do not ordinarily produce visible quantities of excretory ommochromes.

Prepupae of *Tatochila* placed at 37°C or higher become suffused with pink, which carries over into the pupa but fades gradually over several days if the pupa is returned to 25°C. Diseased pupae of *Pieris* (*Synchlœ*) and *Tatochila*, but not *Pieris* (*Artogeia*), often develop a red "saddle" in the mid-dorsum which may spread over the entire body, but not the wing-cases; such pupae never recover and are found to contain a bacterial broth. Pupae of *Euchloe ausonides* and *hyantis* develop a characteristic rose-carmine flush if diseased or attacked by parasitoids. The flush starts in one area and gradually spreads over the abdomen as more tissues become involved. Heat-shocked pupae of Nymphalini which are killed by the treatment generally turn purple-pink. All of these phenomena are suspected to involve abnormal ommochrome synthesis.

Abnormal failure to produce excretory ommochromes is also observed in from less than one percent to five percent of Pierini in mass culture; only the second (colorless) meconium is excreted. This phenomenon is observed both as scattered individuals in large broods and as clusters of sibs from particular families. The implication of genetic variation in the ommochrome pathway suggests caution in using evidence from one or a few reared examples in a typological way for taxonomic purposes. Nonetheless, the usefulness of these pigments in classification and the understanding of their function would benefit if more breeders would record the color, if any, of the final larval feces and meconia of whatever species they have in culture, and publish this information.

*Acknowledgments.* I thank Mr. Marc Tatar for help with the literature. South

American work contributing to this paper was funded by the National Science Foundation (DEB-76-18611), the National Geographic Society, and the UCD Institute of Ecology. Two anonymous reviewers of the paper provided useful suggestions, the Fukuda reference (which is in Japanese), and unpublished information on the distribution of red meconia in some Palearctic genera.

### Literature Cited

- BECKER, E., 1942. Über Eigenschaften, Verbreitung, und die genetischentwicklungs—physiologische Bedeutung der Pigmente der Ommatin—und Ommingruppe (Ommochrome) bei Arthropoden. Z. Vererb. Lehre 80: 157-204.
- BUTENANDT, A., U. SCHIEDT, E. BIEKERT, & P. KORNMANN, 1954. Über Ommochrome. I. Mitteilung: Isolierung von Xanthommatin, Rhodommatin, und Ommatin C aus den Schlupfsekreten von *Vanessa urticae*. Liebigs Ann. Chem. 586: 217-228.
- COURTNEY, S. P., 1980. Studies on the biology of the butterflies *Anthocharis cardamines* (L.) and *Pieris napi* (L.) in relation to speciation in the Pierinae. Unpublished Ph.D. thesis, University of Durham, U.K.
- DeBACH, P., M. ROSE & D. ROSEN, 1978. Biological and systematic studies of developmental stages in *Aphytis*. III. Meconia as a possible systematic tool in *Aphytis*. Hilgardia 46(3): 102-112.
- FUKUDA, H. *et al.*, 1972. *Insects' Life in Japan*. Vol. 3, Butterflies. (in Japanese.) Hoikusha, Osaka. p. 71.
- GEIGER, H. J., 1981. Enzyme electrophoretic studies on the genetic relationships of Pierid butterflies. I. European taxa. J. Res. Lepid. 19: 181-195.
- HOLLAND, W. J., 1898. *The Butterfly Book*. Doubleday, New York. 382 pp.
- HOROWITZ, N. H., 1940. A respiratory pigment from the eggs of a marine worm. Proc. Nat. Acad. Sci. USA 26: 161-163.
- HOROWITZ, N. H. & J. P. BAUMBERGER, 1941. Studies on the respiratory pigment of *Urechis* eggs. J. Biol. Chem. 141: 407-415.
- LAFONT, R. & J. L. PENNETIER, 1975. Uric acid metabolism during pupal-adult development of *Pieris brassicae* L. J. Ins. Physiol. 21: 1323-1336.
- LINZEN, B., 1974. The tryptophan-ommochrome pathway in insects. Adv. Ins. Physiol. 10: 117-246.
- SHAPIRO, A. M., 1980. Convergence in pierine polyphenisms. J. Nat. Hist. 14: 781-802.
- \_\_\_\_\_, 1981. The Pierid red-egg syndrome. Amer. Nat. 117: 276-294.
-